Predators of the nest-making spider mite *Schizotetranychus brevisetosus* (Acari: Tetranychidae)

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ABSTRACT

Several species of spider mites construct silk nests on leaf surfaces wherein they develop and reproduce (web-nesting species). Nesting patterns and behaviour of spider mites are related to predator avoidance, but the study of their influence on predatory fauna has been limited to species of a few genera. The present study investigates the occurrence of predators of *Schizotetranychus brevisetosus*, which make web nests and attack some predators, on evergreen oak in Kochi Prefecture, Japan. Of the total individual predators observed (n = 129), the highest proportion (38%) consisted of rove beetles *Holobus* spp. (Coleoptera: Staphylinidae), which are nest intruders and prey on mites at all stages of development. The second most abundant predators (26%) were *Anystis* spp. (Acari: Anystidae), which capture mites outside the nest. The common predators Phytoseiidae spp. (Acari) accounted for only 15% and were mostly found in hibernation. Other predators were rare, except for egg-eating *Agistemus* spp. (Acari: Stigmaeidae) (13%). The findings suggest that web nests and anti-predatory behaviours may bias the predatory fauna toward species that can cope with these anti-predatory strategies.

Key words: *Anystis baccarum*, evergreen host plant, *Holobus kashmiricus beneficus*, *Quercus glauca*, web-nesting life types

INTRODUCTION

Silk threads spun by spider mites (Acari: Tetranychidae) act as defence against predators in various ways (Lemos et al., 2010; Oku et al., 2003; Saito et al., 2016a; Shimoda et al., 2009; Yano, 2012). Several species from different genera display common characteristic behaviours. For example, several mites of *Schizotetranychus*, *Oligonychus*, and *Stigmaeopsis* construct a densely woven silk nest roofing on the depressions of leaf surfaces or over spaces alongside leaf veins, inside which they develop and reproduce (Saito, 2010). This behaviour is referred to as
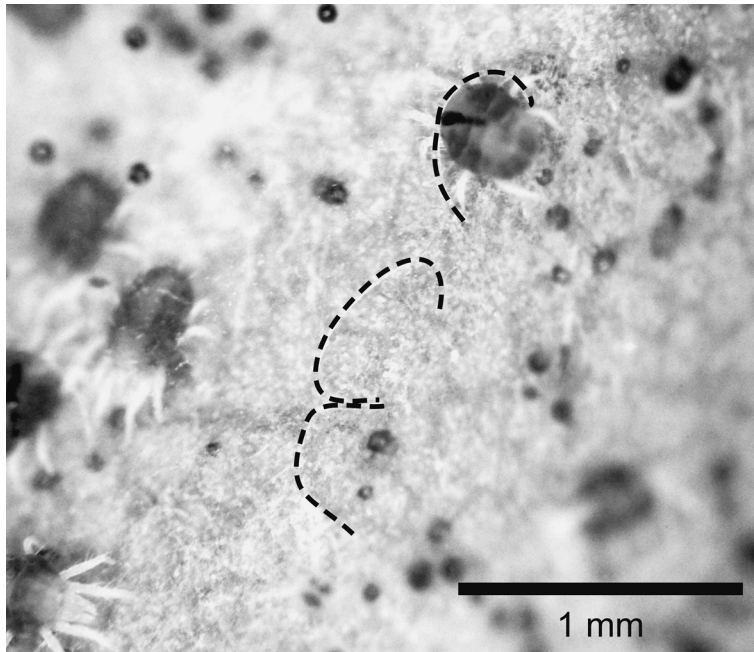
Fig. 1. Pores on a web nest of *S. brevisetosus*. Mites and small predators can pass through these spaces (an adult female is entering). Pore shapes are outlined by dashed lines.

web-nesting (WN), and species demonstrating this behaviour are known as WN species (Saito, 1983; Saito et al., 2016a,b). These nests are themselves a form of predator avoidance, and anti-predatory strategies vary among species that have different web characteristics (Saito, 1983). For example, the nest sizes of *Stigmaeopsis* species are highly variable, and larger nests offer less protection against generalist predators than smaller nests because their larger entrance size allow predators to intrude (Saito et al., 2004, 2016a,b). Some adult *Stigmaeopsis* have been known to drive out or even kill phytoseiid mites that intrude into the nest (Mori et al., 1999; Mori and Saito, 2004, 2005; Saito, 1986a,b) or lock predators out of the nest by covering the nest entrance with silk threads immediately after they detect predator intrusion (Saito and Zhang, 2017). These observations imply that alternative strategies have evolved even among closely related species under disruptive selection pressures imposed by predators (Mori, 2001; Saito, 2010), and predatory fauna might vary depending on the strategies that different species adopt (Mori and Saito, 2004). However, studies have been restricted to the species of *Stigmaeopsis* and few other genera (Horita et al., 2004; Yanagida et al., 2001), and the predator–prey interaction mediated by webs and behaviours is unclear in the entirety of WN species.

    Schizotetranychus brevisetosus Ehara, a WN species (the subtype WN-s, in which mites defecate on the roof; Saito, 1983) phylogenetically distant from *Stigmaeopsis* (Matsuda et al., 2014), is specialised on the evergreen oak, *Quercus glauca* L. (Fagaceae; Ehara, 1989; Ehara and

Gotoh, 2009). All developmental stages live gregariously in a silk-web nest, and adult males engage in lethal fights for mating opportunities with newly emerged females (Masuda et al., 2015). Their webs are less densely woven than those of *Stigmaeopsis*, and sometimes there are large spaces between threads that allow small predators to pass through (Fig. 1). Adult females cover their eggs with silk threads to protect them from predators (Fig. 2.7 in Saito, 2010), whereas adult *Stigmaeopsis* females only spread thin threads under their eggs to affix them to the leaf surface (Kanazawa et al., 2011; Saito, 2010). Although *S. brevisetosus* shows a very low intrinsic rate of natural increase ($r_m = 0.060$ at 25℃; Tamura and Ito, 2017), they attack predators aggressively; for example, adult and nymphs kill predatory midge larvae outside the nest (Ito, 2019). Thus, only the predators that can bypass web protection and avoid strong counterattacks by *S. brevisetosus* can prey on individuals inside the nest.

The composition of developmental stages varies considerably by season (Ito and Yamanishi, 2019). In the central part of Kochi Prefecture, adult females lay white or yellow eggs from spring to autumn (referred to as "summer eggs" in Ito and Hamada, 2018; Ito and Yamanishi, 2019), which hatch soon after deposition. All developmental stages are present in May to October. In autumn, immature mites develop to overwintering adults and they cease oviposition. By late November, the nest population almost exclusively consists of adult gregarious females (males are few because of a heavily biased sex ratio at emergence; Tamura and Ito, 2017). From December to early March, adult females remain active (non-diapausing) and lay bright orange eggs, which are larger than the summer eggs, have robust egg surfaces, and tend to lose egg stipes (referred to as "winter eggs" in Ito and Yamanishi, 2019), and the density of these eggs rises in February. Thus, only these two stages survive the coldest months of the year. The females die out until late March, whereas the winter eggs hatch in early March and finish development in early April, and these next-generation individuals migrate to form a colony on newly expanded leaves. The density of active stages shows two peaks in May and October, and remains low in summer (Ito and Yamanishi, 2019). Such a biannual pattern of egg laying is only known in *Yezonychus sapporensis* Ehara (Saito, 2010) and *Sasanychus akitanus* (Ehara) (Gotoh, 1986a,b) on evergreen *Sasa* bamboos in the northern part of Japan, but its ecological meanings are not sufficiently elucidated.

The present study investigates the seasonal occurrence of predatory insects and mites in Kochi Prefecture from 2014 to 2017, and summarise observations of predatory fauna in the habitat of *S. brevisetosus*. Based on the results, the possible effects of the web nests and anti-predatory behaviours on the predatory fauna are discussed.

MATERIALS AND METHODS

The study was conducted using the roadside trees *of Q. glauca* at Kochi Prefectural Forestry Technology Research Centre in O'hira, Tosayamada, Kami-city, Kochi Prefecture, Japan (33.633°N, 133.708°E, WGS84, 96 m ASL; Geospatial Information Authority of Japan, 2018).

The study was conducted from October 2014 to June 2017. Three to twenty-six leaves infested with *S. brevisetosus* were sampled from approximately ten trees on a monthly basis between 6 October 2014 and 7 February 2016. However, predators were often absent in monthly monitoring

and their activities could not be followed, because they rapidly destroyed the mite colonies and moved away. Therefore, the sampling intervals were shortened to once to thrice per month until 22 June 2017. The branches for sampling were arbitrarily selected every time, because the mite distribution on the tree was frequently changed by predation or dispersal to other leaves.

The sampled leaves were individually packed in plastic bags and placed in a biomedical freezer (−30℃, MDF-236; Sanyo, Osaka, Japan) for fixation within 24 h of collection. Individual predators in feeding stages were counted using a stereomicroscope (SZX7; Olympus, Tokyo, Japan), and were identified following Shimoda et al. (1993a), Ehara and Shinkaji (1996), and Ehara and Gotoh (2009).

Because predators were sometimes found on leaves distant from the *S. brevisetosus* colony, we also performed on-site counting of predators on the leaves of adjacent branching stems, using a magnifying glass (14×, NKL-14; Winner, Chiba, Japan). Based on the density of *S. brevisetosus* (the number of individuals per infested leaf; Ito and Yamanishi, 2019), the number of leaves monitored varied from 100–1,200. Predator density, defined as the number of individual predators (including those on adjacent stems) averaged over infested leaves, was calculated for each survey date. Because not all predators could be precisely identified on site, several predators were summarised at the family or genus level.

RESULTS

The seasonal occurrence of each predator is summarised in Table 1 and Fig. 2. Predators were observed until 5 December in 2014. In 2015 and 2016, predatory activities were observed from 10 May to 4 December and from 2 March to 19 December, respectively. Predators were first observed on 5 April in 2017. No active predator was found in January and February in any year, though there were the winter eggs and adult females of *S. brevisetosus* on the leaves (Table 1).

Predatory rove beetles *Holobus* spp. (Coleoptera) accounted for 38% of the predators, and they mostly comprised the common species *Holobus kashimiricus beneficus* (Naomi), which is synonymous with *Oligota kashmirica benefica* Naomi (Kanao et al., 2016; Naomi, 1984). *Holobus* spp. were found inside and outside the nest of *S. brevisetosus*, but preyed on all stages of *S. brevisetosus* mainly in the nest. Their density peaked twice on 20 May and 9 September 2016. The autumnal peak comprised 23 immature individuals and one adult from 300 assessed leaves, resulting in the highest predator density recorded in this study. Their density steeply declined after the second peak, and only one individual was recorded every month from October to December (Table 1, Fig. 2a). The peak density and the timing of the peaks fluctuated yearly (Fig. 2); smaller peaks occurred in 24 May 2017 and 4 October 2015 but without second peak in either year.

Anystis baccarum L. (Acari: Anystidae), which run very fast on plants and capture tiny arthropods encountered, accounted for 26% of the total detected predators. Similarly to *Holobus* spp., they were absent in winter, and their density peaked on 3 May 2016. The number of *Anystis* from 20 April 2016 to 1 June 2016 accounted for 38% (n = 34) of the total number of *Anystis* observed throughout the entire study. No conspicuous peaks were observed in other years (Table 1; Fig. 2a). Unlike other predators, *Anystis* mites were unable to enter *S. brevisetosus* nests

Table 1. Temporal distribution of individual predators (n = 129 in total) in the study patch of *Schizotetranychus brevisetosus* per month in Kami, Kochi Prefecture. Data are cumulated over October 2014 to June 2017.

Order	Genus/Family	%	J	F	M	A	M	J	J	A	S	O	N	D
Coleoptera	*Holobus*	38					15	2		1	24	4	2	1
Acari	*Anystis*	26				4	9	4	3	1	2	8	2	1
Acari	Phytoseiidae	15	10[*]		2		1	3					1[*]	2[*]
Acari	*Agistemus*	12			1	6	1	4			1			3
Diptera	*Feltiella*	7									1	2	1	5
Thysanoptera	*Scolothrips*	1										1		
Coleoptera	*Stethorus*	1								1				

[*]Hibernating *Amblyseius eharai* adults

because they have a large body (1.0–1.5 mm in length; Cuthbertson and Murchie, 2010), and are therefore easily hampered by silk webs. Instead, they captured *S. brevisetosus* walking on the leaf surface or those climbing up the nest roof to defecate.

Phytoseiid mites (Acari: Phytoseiidae) accounted for 15% of the total number of predators (Table 1, n = 19). The only identified species of phytoseiid mites was *Amblyseius eharai* Amitai et Swirski. The density of active phytoseiid mites did not appear to peak in either year, with a few active individuals occasionally found between 7 March and 28 June 2016 (Table 1; Fig. 2b). Thirteen individuals collected from November to January (68%, n = 19) were all adults overwintering in the deserted silk webs of spiders (*Myrmarachne* spp.) or in the empty leaf rolls constructed by the larvae of the leaf-rolling moth *Povolnya querci* (Kumata) (Gracillariidae).

Egg-feeding *Agistemus* sp. (Acari: Stigmaeidae) sometimes occurred in the colonies on new leaves, primarily between 22 March and 14 June 2016 (75%, n = 16; Fig. 2b). The remaining types of predators were observed much less frequently (Table 1, Fig. 2b). The larvae of *Feltiella acarisuga* (Vellot) (Diptera) (n = 9) were occasionally found in the web from September to December in 2015 (78%) and December in 2016 (22%). The thrips *Scolothrips takahashii* Priesner (Thysanoptera) and the lady beetle *Stethorus japonicas* Kamiya (Coleoptera) were found only once on 6 October 2014 and 17 August 2015, respectively.

DISCUSSION

The overall predator abundance showed two seasonal peaks in spring and autumn (Table 1),
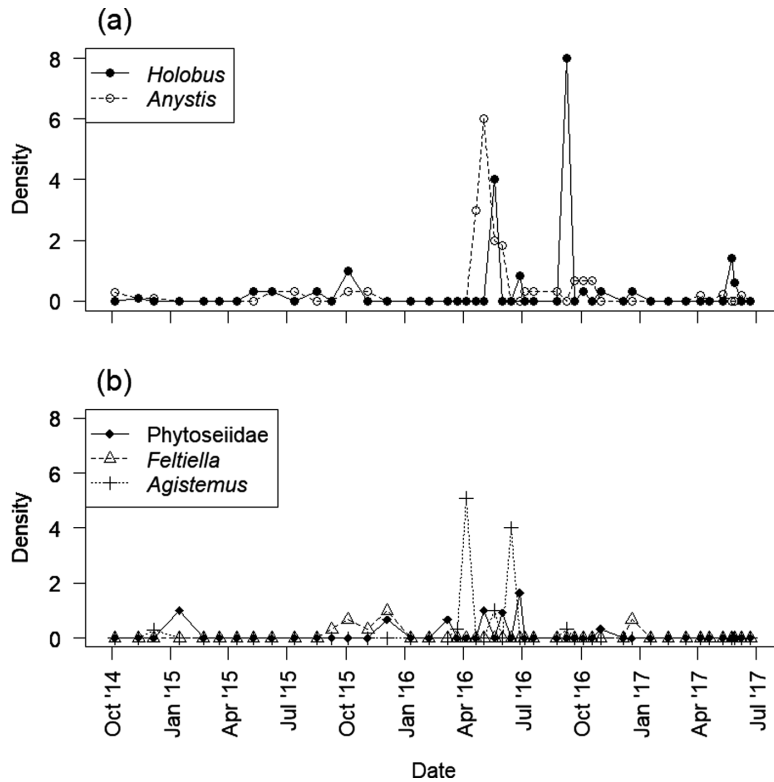
Fig. 2. Predator density per 100 leaves of *Quercus glauca* in Kami, Kochi Prefecture
from October 2014 to June 2017 (n = 129). The horizontal axis label indicates the
first day of the month and the two-digit year. (a) Rove beetles (*Holobus* spp.) and
Anystis mites; (b) Phytoseiid mites, *Agistemus* mites, and predatory midge larvae
(*Agistemus* sp.).

which were especially apparent in 2016. The high density in 2016 may be related with the density
of mites in the same year. Ito and Yamanishi (2019) showed that the field density of *S.
brevisetosus* peaked in almost the same seasons. Thus, these predators are likely to depend on *S.
brevisetosus* as a major food source. Although all predators observed here are ubiquitous in the
temperate region of Japan (Ehara and Gotoh, 2009), their composition is characterised by a small
fraction of phytoseiid mites, with larger fractions of the rove beetles and *Anystis* mites. Predator
compositions among WN species have been studied much less than those of non-WN species
such as *Tetranychus* spp., of which phytoseiid mites are major predators (e.g., Hamamura, 1986;
McMurtry and Croft, 1997; Takafuji and Chant, 1976). Several phytoseiid species are also known
to be predominant predators of the WN species (Aponte and McMurty, 1997; Gotoh, 1987;
Kropczynskam et al., 1988; Saito, 1990; Takano-Lee and Hoddle, 2002; Yanar and Ecevit, 2008).
However, although a simple comparison is difficult, the density of phytoseiid mites in the present
study of *S. brevisetosus* appears to be by far the lowest reported for any WN species. For
example, Shimazaki et al. (2019) frequently observed more than 0.5 phytoseiid mites per leaf on
each survey date for *Oligonychus castaneae* Ehara and Gotoh populations on chestnut trees.

Thus, such a low density of phytoseiid mites might be related with the anti-predatory strategies of *S. brevisetosus*. In the following section, the ecological characteristics of these predators are summarized.

Holobus

The rove beetles *Holobus* spp. consume numerous individuals of spider mites over a short term (Kishimoto, 2003; Kishimoto and Adachi, 2008). Immature *H. k. beneficus* (previously referred to as *Oligota kashmirica benefica* Naomi) preyed on several hundreds of *Tetranychus urticae* eggs to finish development (Shimoda et al., 1993b). In the present study, *Holobus* spp. were the most abundant predators, representing 38% of the total predator population (Table 1), and most of them were *H. k. beneficus*, which is a widespread native predator specialised on spider mites. Their density showed two seasonal peaks in May and October in 2016 (Fig. 2a), which ~~generally~~ corresponded with the time when the number of summer eggs and hatchlings of *S. brevisetosus* are increasing (Ito and Yamanishi, 2019). A similar seasonal trend with two density peaks is also reported in *H. k. beneficus* feeding on *Tetranychus* mites on the kudzu vines (Shimoda et al., 1993b), which often grow near *Q. glauca* trees in the study site. The density peaks of *Holobus* spp. were obscure in 2015, even though *S. brevisetosus* were at a high density in spring from late March to early May (nearly 500 eggs per infested leaf; Ito and Yamanishi, 2019). It is possible that *Holobus* spp. destroyed the mite colonies within a few weeks, which would mean that their activity would not have been detected by monthly monitoring. Adult *Holobus* spp. can migrate across distant patches (Shimoda et al., 1993b), and Shimoda and Takabayashi (2001) demonstrated that the settlement of *H. k. beneficus* in the mite patch is cued by volatiles emitted from *T. urticae*-infested bean leaves. If *Holobus* spp. are also able to perceive the volatiles from oak leaves infested with *S. brevisetosus*, they are likely to migrate to the habitats of *Tetranychus* mites (i.e., weeds such as kudzu vines or cultivated crops) when *S. brevisetosus* is depleted, and return to the *S. brevisetosus* patches after *Tetranychus* mites are consumed. The movement of *Holobus* spp. should be clarified to understand their effects on the population dynamics of *S. brevisetosus*.

~~As far as I observed,~~ active *Holobus* individuals sampled from the study site and then placed on the leaf culture of *S. brevisetosus* ~~in the laboratory~~ easily invaded the nests and consumed the mites at all developmental stages, including eggs covered by protective silk threads. Mites did not counterattack intruding *Holobus*, probably because the robust cuticles of *Holobus* are highly protective. ~~In addition, *Holobus* easily broke into the nest with their mandibles.~~ Thus, neither web-related nor behavioural defences are considered ineffective. However, *S. brevisetosus* nymphs and adults in the field were observed to migrate to nearby leaves as a group when their nest was disturbed~~, which~~ could play an anti-predatory role~~.~~ This prediction should be tested in the further studies.

Anystis

The second major group was the whirligig mite *Anystis* sp., which preys on various phytophagous arthropods (Cuthbertson and Murchie, 2004; 2010). *Anystis* sp. were observed from April to December (Table 1), and showed a large peak in spring and a small peak in autumn (Fig. 2a). Such patterns, particularly the large peak in spring, have been reported in orchards (Cuthbertson and Murchie, 2010) and in tea fields (Ishikawa, 1977). Cuthbertson and Murchie

(2004) highlighted that the spring peak in the orchard coincides with the time when spider mites migrate to new foraging areas and reproduce. In the present study, the spring peak from late April to early May in 2016 similarly coincided with the period when *S. brevisetosus* established colonies on new leaves (Ito and Yamanishi, 2019). Thus, *S. brevisetosus* may be an important food resource for *Anystis* mites in spring. *Anystis* mites were unable to enter *S. brevisetosus* nests as they were unable to bypass the silk webs (Sorensen et al., 1976), and they only fed on *S. brevisetosus* at active stages that they encountered outside their nests. Thus, despite being the second-most abundant predatory species, they exerted far less predation pressure than *Holobus* spp. Nevertheless, interactions between different types of predation, as shown by *Holobus* spp. (predation inside the nest) and *Anystis* spp. (predation outside the nest), are an important subject of study in community ecology (Otsuki and Yano, 2014).

Phytoseiidae

Phytoseiid mites, which are usually dominant in the habitat of spider mites in various crops (Helle and Sabelis, 1985), notably represented only 15% of the total predators and showed no apparent seasonal trend (Table 1, Fig. 2b). Their active stages were found only from March to June, when *S. brevisetosus* colonies were established on new leaves (Ito and Yamanishi, 2019). The sole identified species was *A. eharai*, which were easily hampered by silk threads spun by spider mites (Ehara and Gotoh, 2009). Considering that only 32% of all phytoseiid mites were found active (Table 1), they therefore impose very low predation pressure on *S. brevisetosus*. The reason for such a low density is unknown, but counterattack from *S. brevisetosus* is a possibility because high densities of phytoseiids are observed in the spider mites that do not show counterattacks (Helle and Sabelis, 1985). Ito (2019) reported that *S. brevisetosus* at both the adult and the developing stages use their legs to capture predatory midge larvae and kill them by sucking out their body fluids, though whether these larvae are a food source for mites is unexplored. More recently, we observed that adult females drove out the thrips *S. takahashii* from their nest by pinching them with chelicerae (Y. Ioku and K. Ito, unpublished data). Because such counterattacks may increase the survival of siblings in the nest, the idea that adults also attack phytoseiid mites is a possibility that is worth investigating.

The reason why predators were not observed in winter is currently unknown. One possibility is that low temperature decreased the activity of predators, but the conditions were not lethal even in the coldest month (the daily mean temperature 7.1–7.2℃ in January at Kochi, Kochi Prefecture; Japan Meteorological Agency, 2020), and temperatures sometimes rise to more than 10℃ that may be enough for predators to act. Another possibility is that the life style of *S. brevisetosus* in winter is defensive. For example, adult populations densely formed on the leaves before oviposition might prevent intrusion of predators in the nest. In addition, these females cover their eggs with numerous silk threads (K. Ito, personal observation), and the surfaces of eggs themselves are robust in winter (Ito and Yamanishi, 1993). The effects of these characteristics should be explored in the further studies.

Conclusion

The WN species construct protective webs and display various anti-predatory behaviours that strongly affect the predator–prey interactions on the leaf surface (Faraji et al., 2001; 2002). The present results suggest that these protective mechanisms affect each predator species, as inferred

by the unusual bias toward rove beetles and *Anystis* mites among the predatory fauna. The WN species are good model species ~~in which the experimental evaluation of~~ the effects of webs and behaviours ~~can be performed separately~~ (Mori et al., 1999; Mori and Saito, 2004; 2005). Further investigation of these effects in more species should be performed to discriminate the factors that affect predator–prey interactions.

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摘要

造巣性のカシノキマタハダニ（ダニ目：ハダニ科）の捕食者について
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　いくつかの種のハダニは葉の表面に絹糸で巣を作り，その内部で繁殖する（WN種）．それらの営巣パターンは捕食回避と関係すると考えられるが，捕食者相に与える影響の検証例はスゴモリハダニ属などの少数の種に限られている．本研究では，高知県香美市において，常緑性のアラカシに寄生するカシノキマタハダニ（*Schizotetranychus brevisetosus* Ehara）の捕食者相を調査した．発見した個体（n＝129）のうち，ハネカクシ類が38％，ハモリダニ類が26％となり両者で全体の半数を占めた．一方，ハダニのパッチでよく見られるカブリダニ科は全体の15％に過ぎず，そのうち多くが越冬中であった．また，卵捕食者のナガヒシダニ科（13％）を除き，他の捕食者はまばらであった．カシノキマタハダニは冬の間も成虫と卵が葉の上に残るが，1月と2月に活動する捕食者はいなかった．これまでの研究結果を踏まえると，本種の巣網や防衛行動が捕食者ごとに異なる効果を及ぼした結果，捕食者相が他種と異なった可能性がある．